

AMENDMENTS IN THE SPECIFICATION:

Page 16, Line 27 (Paragraph beginning thereat):

a position corresponding to position 4 in the motif sequence 2L: A-L-G-N-G-G-L-G; and

Page 17, Line 28 (Paragraph beginning thereat):

In one embodiment, the α -glucan phosphorylase having improved thermostability has an amino acid residue which is different from an amino acid residue of the natural α -glucan phosphorylase at a position corresponding to phenylalanine at position 39 (MF39); or a position corresponding to threonine at position 706 (T706) in an amino acid sequence of SEQ ID NO: 2.

Page 32, Line 18 (Paragraph beginning thereat):

For example, according to Toshio Fukui, et al., Biochemistry of Vitamin B6, 1987, pp.267-276, the Km (Michaelis constant) of potato leaf-derived type L α -glucan phosphorylase for glycogen is 1.4×10^{-3} (M), while the Km of potato leaf-derived type H α -glucan phosphorylase for production of glycogen is 4×10^{-6} (M). In addition, the Km of a main component of potato tuber-derived α -glucan phosphorylase for production of glycogen is 2.4×10^{-3} (M), and this is classified as type L. The Km of a minor component α -glucan phosphorylase for production of glycogen is 1×10^{-6} (M), and this is classified as type H.

Page 81, Line 19 (Paragraph beginning thereat):

In the enzyme according to the present invention, an amino acid residue at a

position corresponding to position 7 or T706 in the motif sequence 3L or 3H can be an amino acid other than an amino acid residue found in natural α -glucan phosphorylase. An amino acid residue at a position corresponding to position 7 or T706 in a motif sequence 3L or 3H is preferably an aliphatic amino acid, more preferably a branched amino acid (i.e. valine, leucine or isoleucine) or a sulfur-containing amino acid (i.e. cysteine, ~~cysteine~~ cystine, methionine), particularly preferably cysteine, isoleucine, leucine, valine or tryptophan, particularly preferably cysteine, isoleucine, leucine or valine, most preferably isoleucine.

Page 81, Line 33 (Paragraph beginning thereat):

In the method according to the present invention, for preparing α -glucan phosphorylase having improved thermostability, a substitution, addition, deletion or modification of an amino acid can be performed in addition to alteration of the object of the invention (such the substitution that an α -glucan phosphorylase has an amino acid residue which is different from an amino acid residue of the natural α -glucan phosphorylase in at least one position selected from the group consisting of a position corresponding to phenylalanine at position 39 (F39) of the amino acid sequence set forth in SEQ ID NO: 2, a position corresponding to asparagine at position 135 (N135) of the amino acid sequence set forth in SEQ ID NO: 2 and a position corresponding to position 706 threonine (T706) of the amino acid sequence set forth in SEQ ID NO: 2). Substitution of an amino acid refers to substitution of one amino acid with another one amino acid. Addition of an amino acid refers to insertion of one or more, for example, 1 to 10, preferably 1 to 5, more preferably 1 to 3 amino acids into any position of the original amino acid sequence. Deletion of an amino acid refers to removal of one or more, for example, 1 to 10, preferably 1 to 5, more preferably 1 to 3 amino acids from the original amino acid sequence. Examples of amino acid modification include but are not limited to amidation, carboxylation, sulfation, halogenation, alkylation, glycosylation, phosphorylation, hydroxylation, and acylation (e.g. acetylation). The α -glucan phosphorylase having improved thermostability of the present invention may be

synthesized by a peptide synthesis method and, in such the case, an amino acid to be substituted or added may be a natural amino acid, a non-natural amino acid or an amino acid analog. A natural amino acid is preferable.

Page 109, Line 1 (Paragraph beginning thereat):

Then, the reaction solution is heated, if necessary, by the method known in the art, to react it. A reaction temperature can be any temperature as long as the effect of the invention is obtained. A reaction temperature can be representatively about 30°C to about 75 °C. It is preferable that the temperature of a solution in this reaction step is such a temperature that activity which is about 20% or more, more preferably about 30% or more of activity of α -glucan phosphorylase contained in this solution before a reaction, remains after a predetermined reaction time. This temperature is preferably about 55°C to about 75 °C, more preferably about ~~60°C~~ 65°C to about 75 °C, further preferably about 60°C to about 70 °C, particularly preferably about 60°C to about 65°C.

Page 126, Line 8 (Paragraph beginning thereat):

PCR was performed to amplify an Arabidopsis thaliana-derived type H GP gene. The conditions of the PCR reaction were 30 cycles, one cycle being 94°C for 30 seconds, 60°C for 1 minute, and 72 °C for 3 minutes. The underlined part of PCR ~~primer 4~~ primer 5 corresponds to a structural gene at the N-terminal region of a mature protein of an Arabidopsis thaliana-derived type H GP gene, and the underlined part of PCR ~~primer 2~~ primer 6 corresponds to a structural gene at the C-terminal region of a mature protein of an Arabidopsis thaliana-derived type H GP gene.

Page 134, Line 1 (Paragraph beginning thereat):

In the above Table 7, WT indicates natural potato-derived type L α -glucan phosphorylase. In each ~~row~~ column, an amino acid represented by one letter

abbreviation indicates an amino acid substituted in a modified GP. For example, an entity expressed by I in a row column labeled with F39 at a left end indicates modified GP in which phenylalanine (F) at position 39 is substituted with isoleucine (I). This is also true for modified GP in other rows columns.

Page 135, Line 26 (Paragraph beginning thereat):

Seeing remaining activity after incubation at 65°C for 2 minutes, when phenylalanine at position 39 was substituted with isoleucine, leucine or valine, thermostability of modified GP was superior to that of natural potato-derived type L GP. Regarding substitution at position 39, substitution with leucine (remaining activity after incubation at 65°C for 2 minutes is ~~64.2%~~ 40.2%) was most excellent with respect to thermostability. When asparagine at position 135 was substituted with alanine, cysteine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, serine, threonine, valine or tyrosine, thermostability of modified GP was superior to that of natural potato-derived type L GP. Regarding substitution at position 135, substitution with alanine (remaining activity after incubation at 65°C for 2 minutes is 79.0%), cysteine (remaining activity after incubation at 65°C for 2 minutes is 76.9%), glycine (remaining activity after incubation at 65°C for 2 minutes is 58.4%), methionine (remaining activity after incubation at 65°C for 2 minutes is 52.6%), serine (remaining activity after incubation at 65°C for 2 minutes is 86.5), threonine (remaining activity after incubation at 65°C for 2 minutes is 62.4%) or valine (remaining activity after incubation at 65°C for 2 minutes is 79.3%) was particularly excellent with respect to thermostability. When threonine at position 706 was substituted with cysteine, isoleucine, leucine, valine or tryptophan, thermostability of modified GP was superior to that of natural potato-derived type L GP. Regarding substitution at a position 706, substitution with leucine (remaining activity after incubation at 65°C for 2 minutes is 57.8%) or valine (remaining activity after incubation at 65°C for 2 minutes is 59.2%) was particularly excellent with respect to thermostability.

Page 137, Line 33 (Paragraph beginning thereat):

0.2U/ml of a purified enzyme solution (20 mM citrate buffer (pH 6.7)) was incubated at 58°C for 10 minutes, 60°C for 10 minutes or 65°C for 2 minutes, and retained on ice. An enzyme solution retained on ice was 10-fold diluted with a 20 mM citrate buffer (pH 6.7), and enzyme activity was measured according to the activity measuring method described in Example 3-1 (2). Thermostability of an enzyme was judged by a ratio of enzyme activity at 37°C of the enzyme after incubation (i.e. remaining activity) when enzyme activity at 37°C of the enzyme before incubation at 58°C for 10 minutes, 60°C for 10 minutes or 65°C for 2 minutes is taken to be 100%. Results regarding potato type H GP having improved thermostability and natural potato type H GP are shown in the following Tables 8 and Fig. 11. Results regarding Arabidopsis thaliana type H GP having improved thermostability and natural Arabidopsis thaliana type H GP are shown in the following Tables 9 and Fig. 12.